

1st Round: screening of mutants (full length Ala-scan)

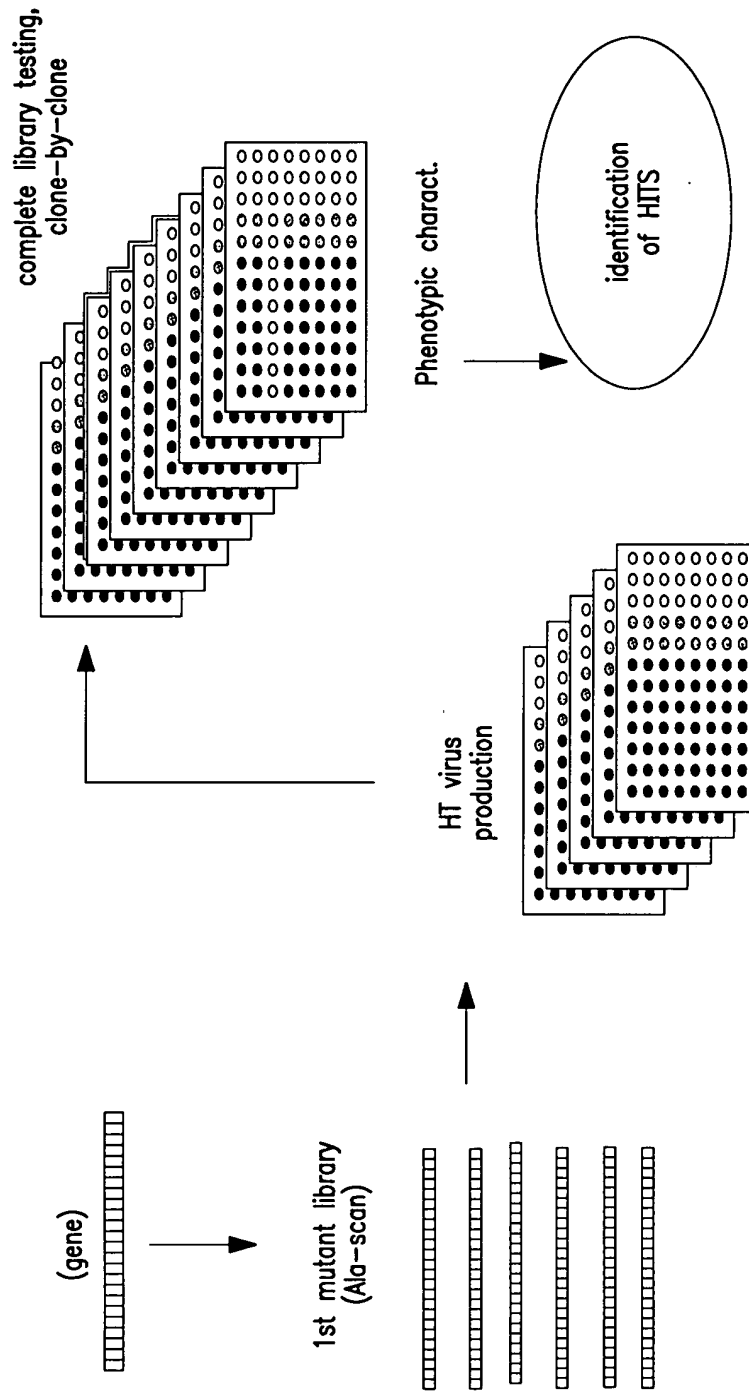


FIG. 1A

2nd Round: screening of mutants at (surrounding) HIT positions

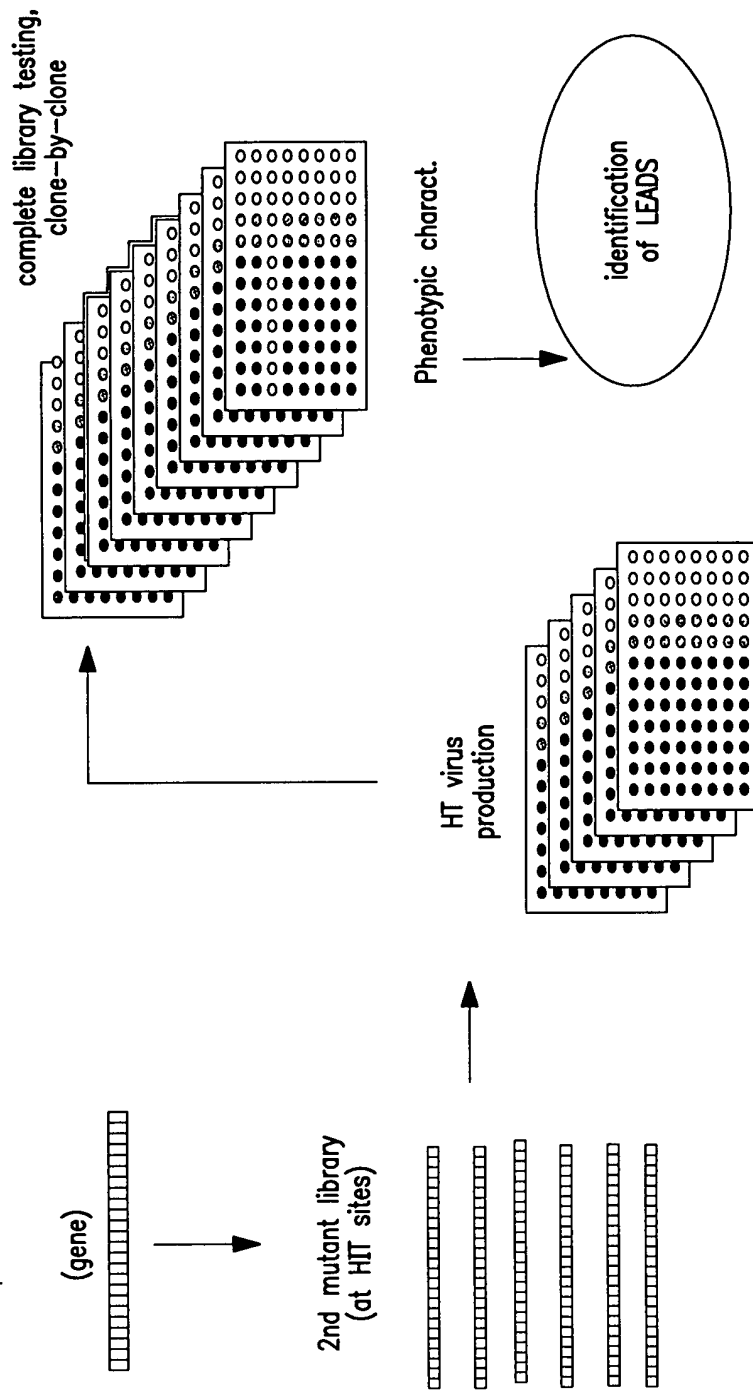


FIG. 1B

3rd Round: screening of recombinants between LEADS

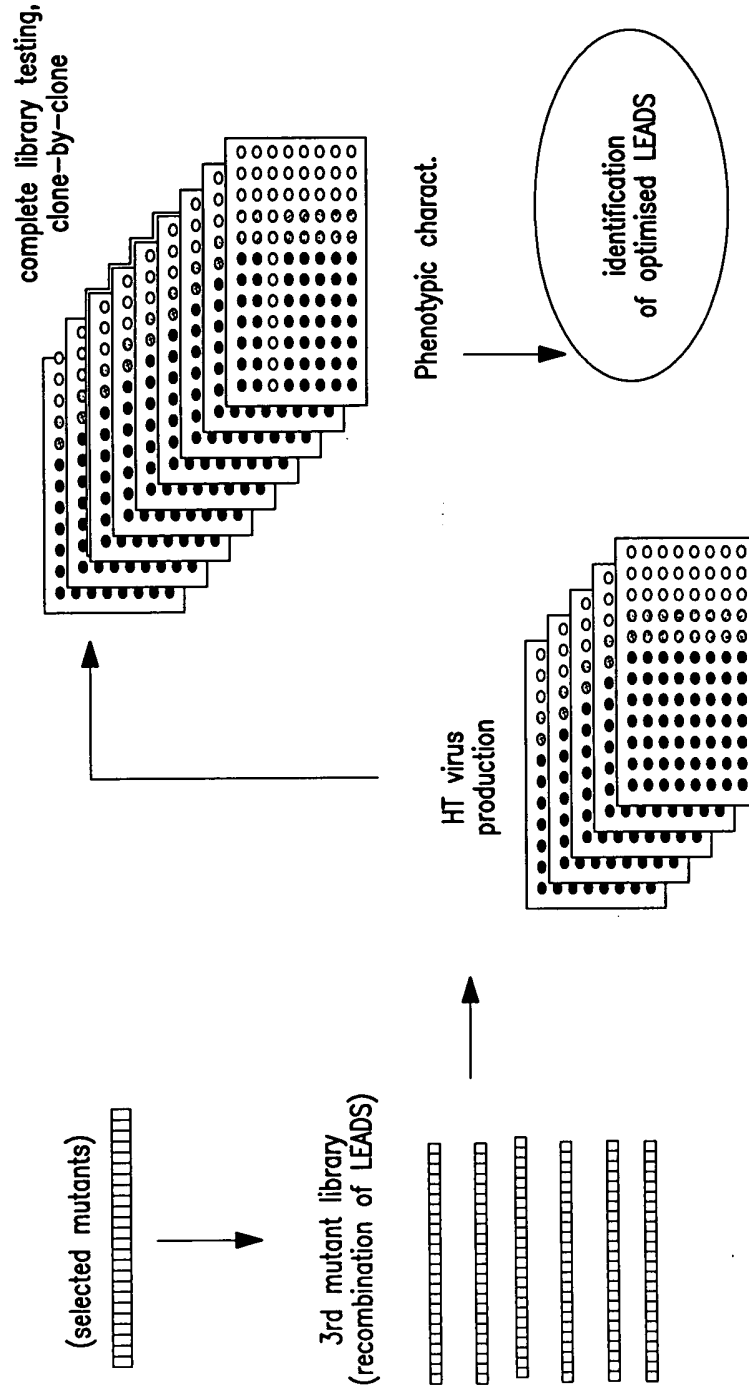


FIG. 1C

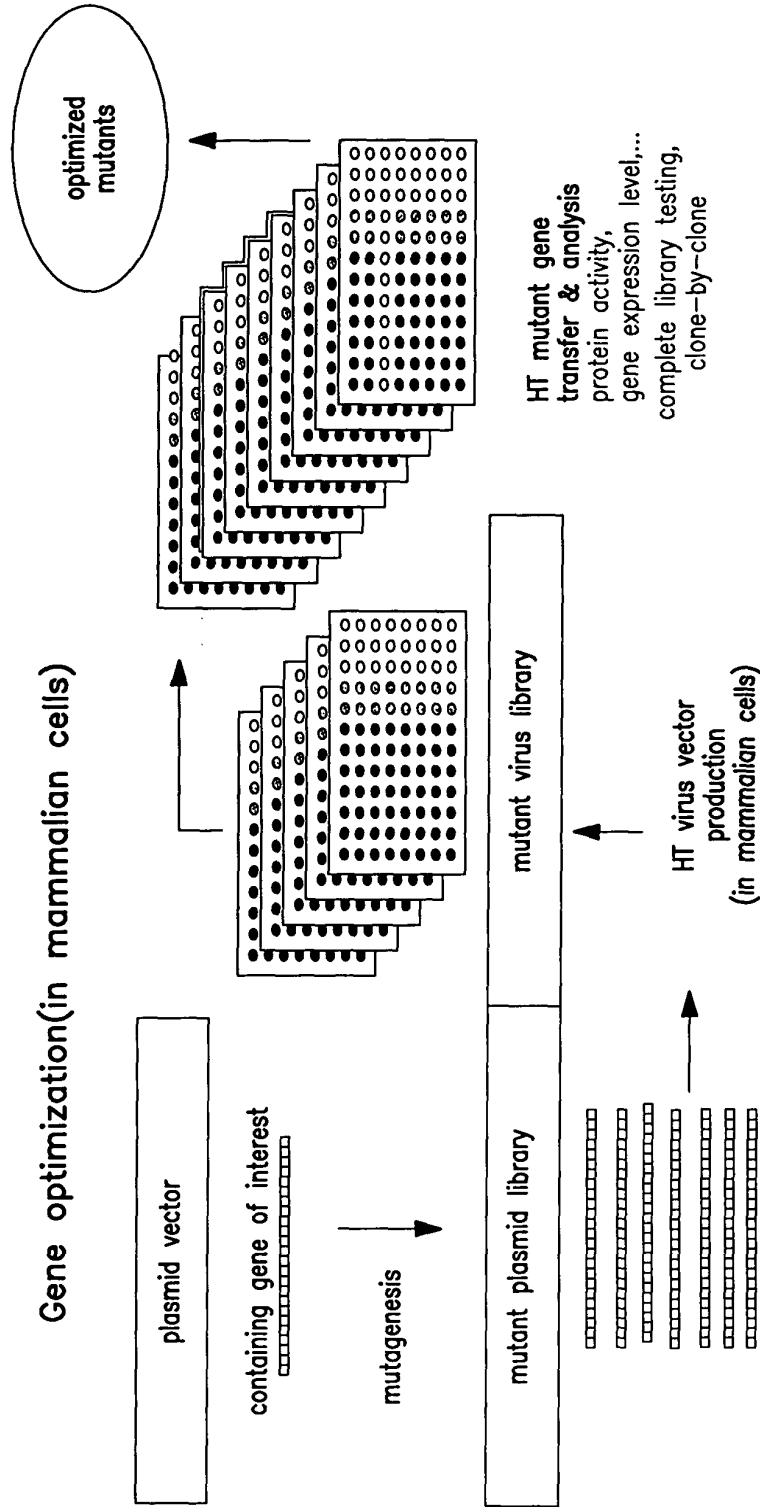


FIG. 1D

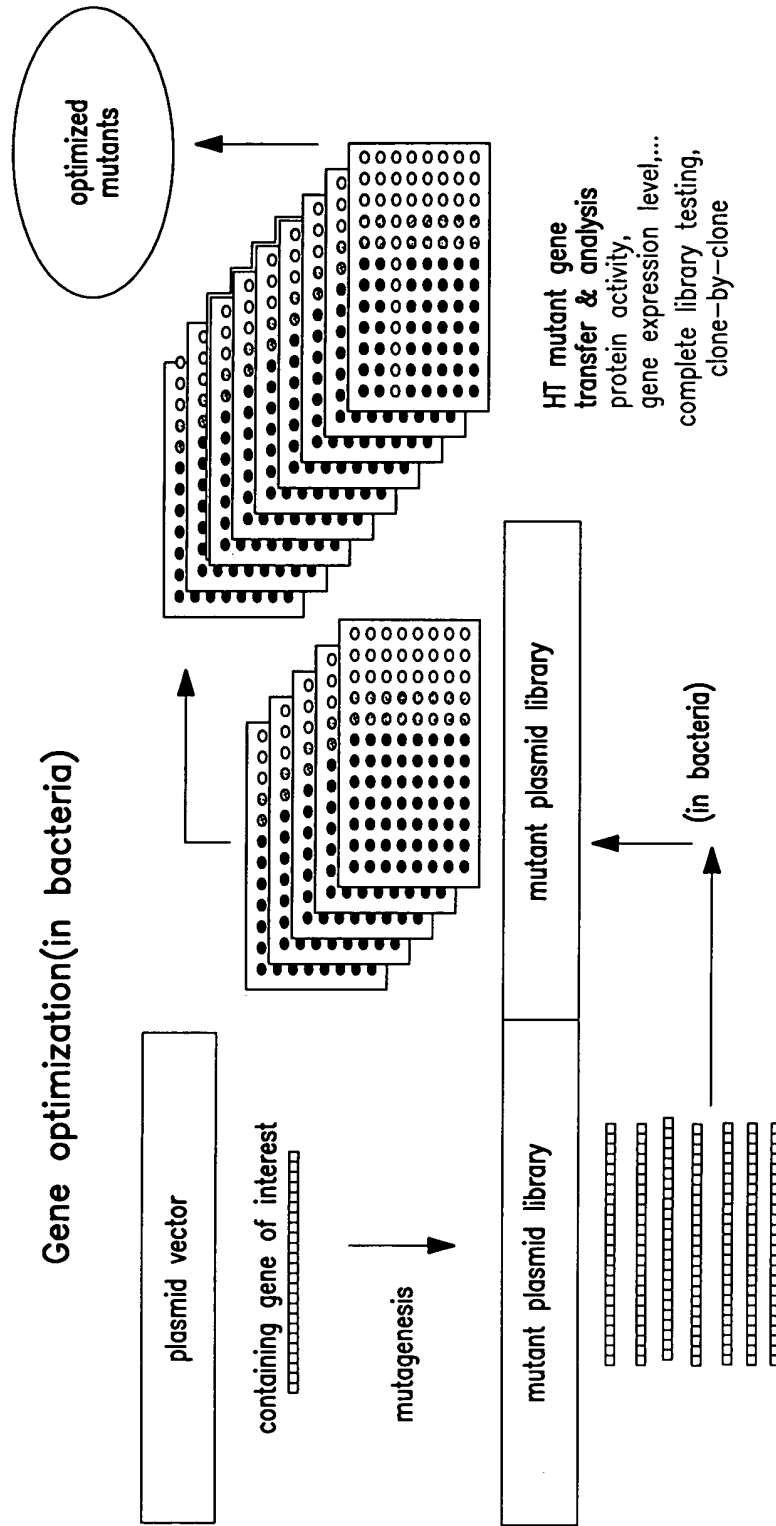


FIG. IE

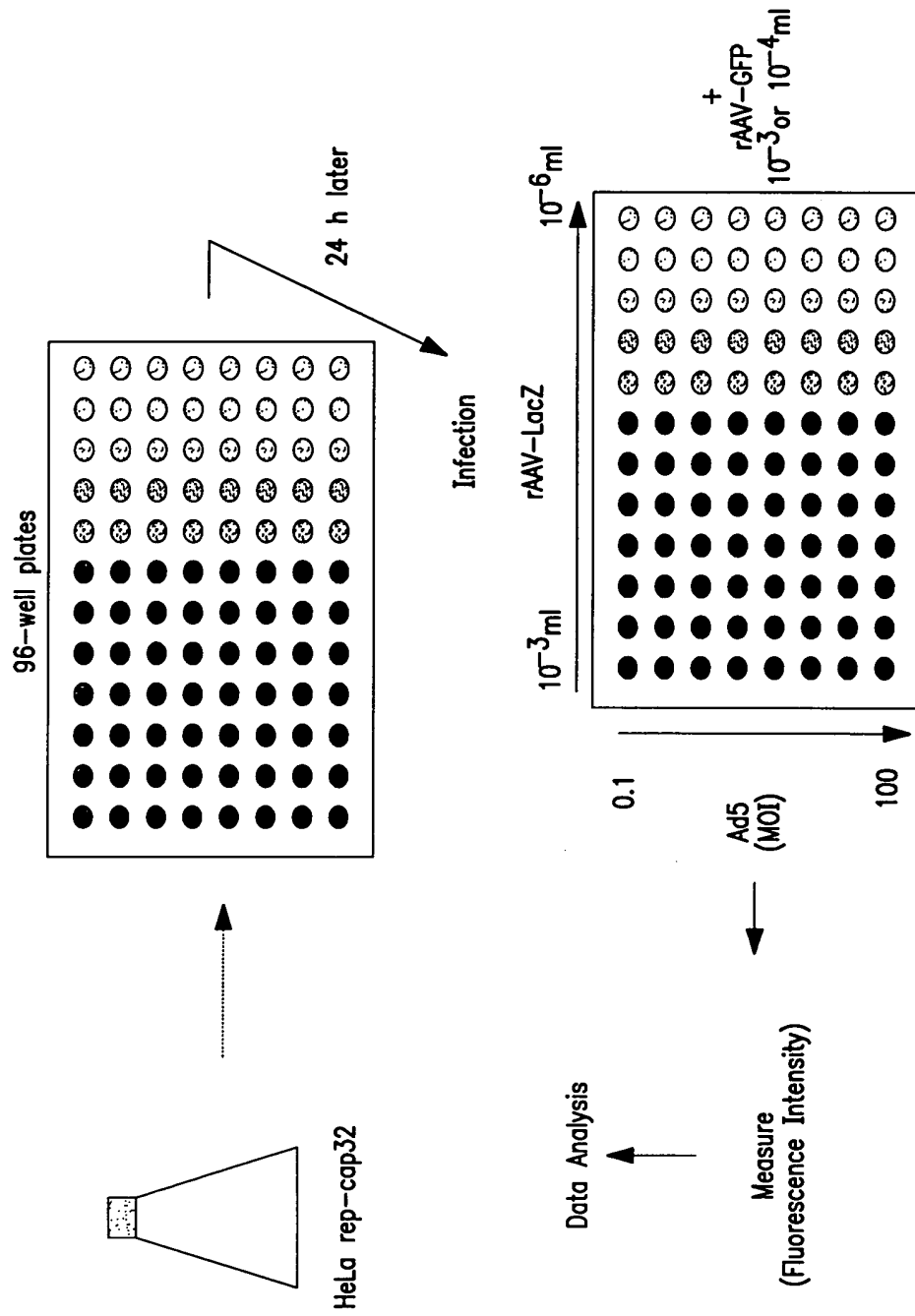


FIG. 2A

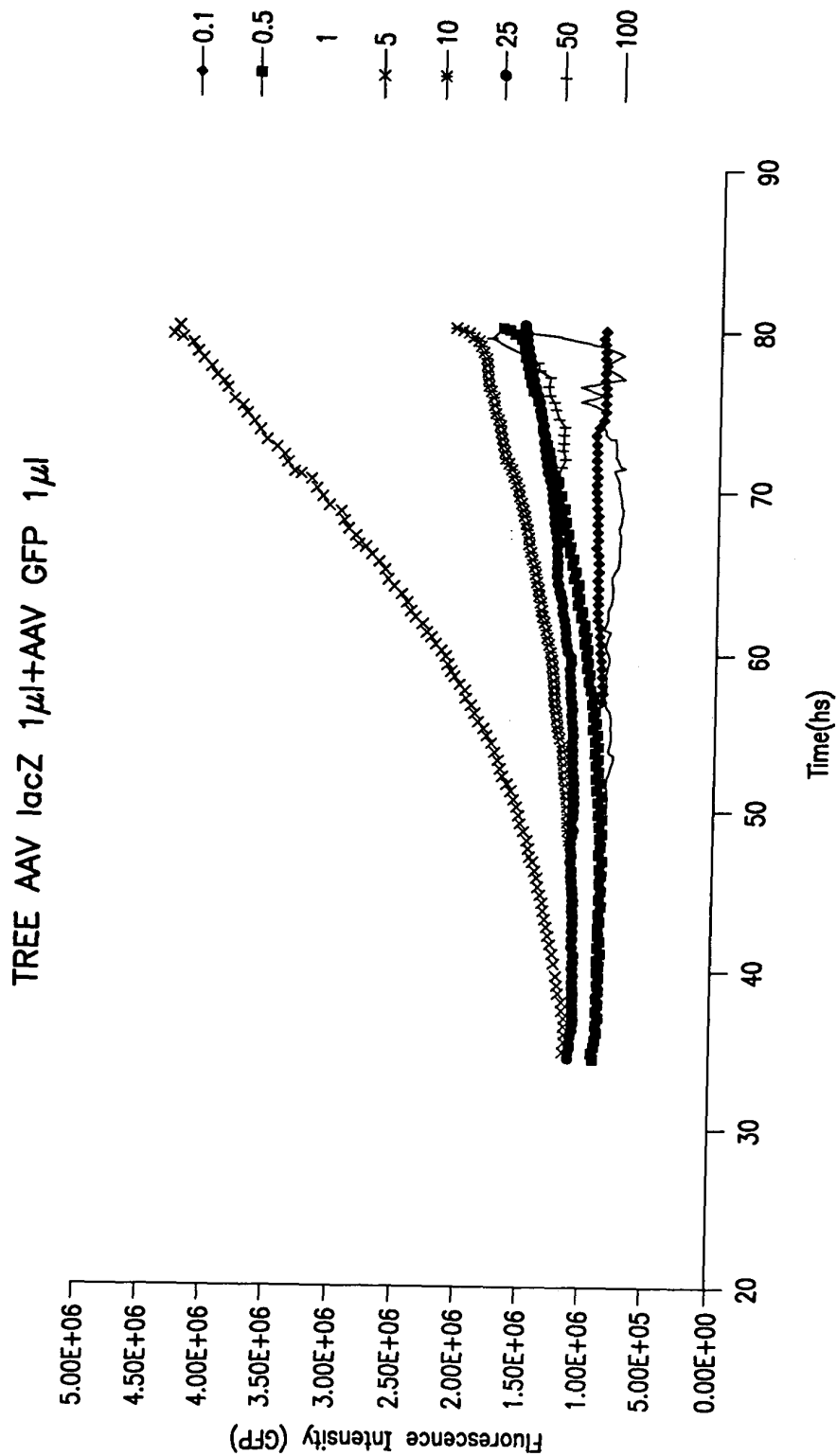


FIG. 2B

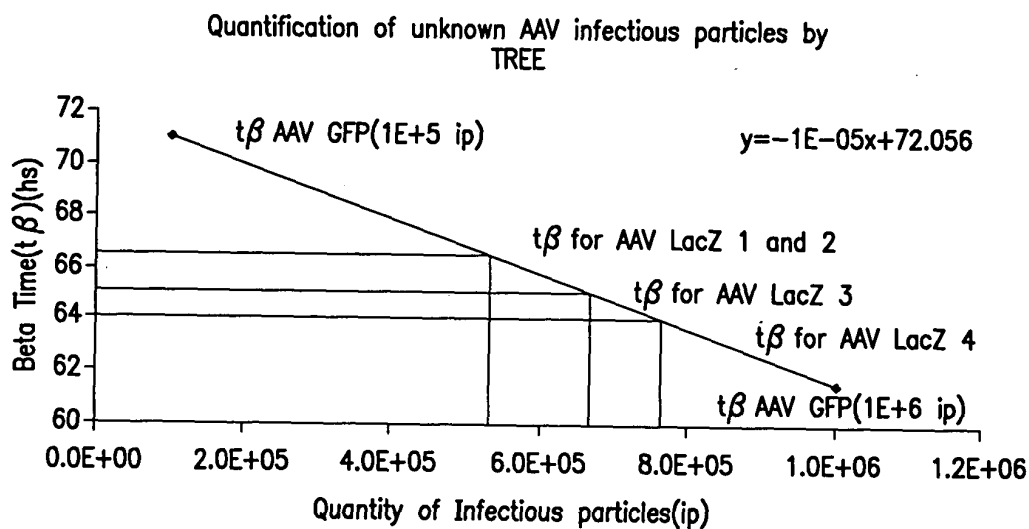


FIG. 2C

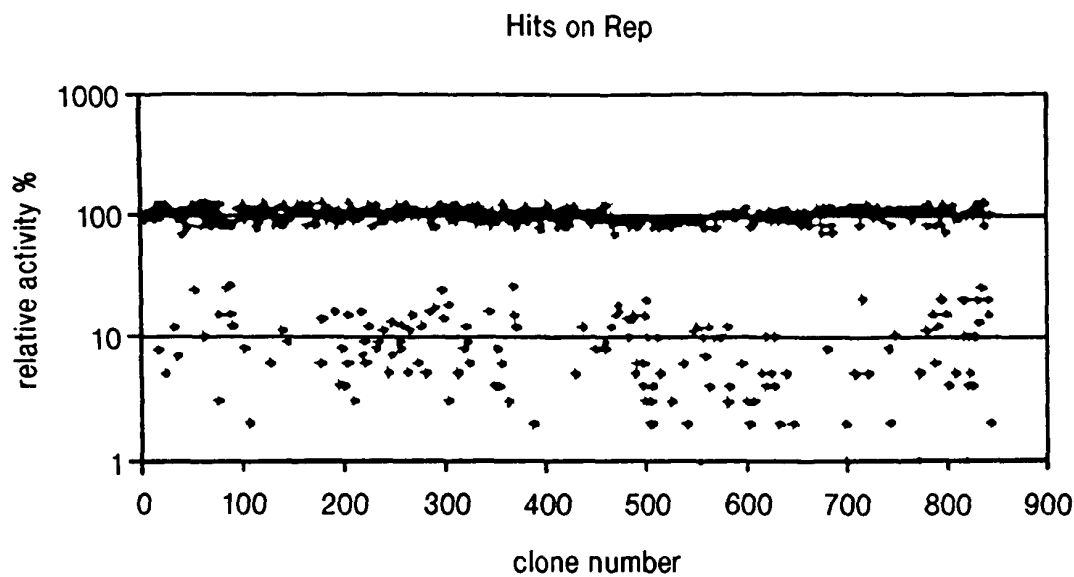


FIG. 3A

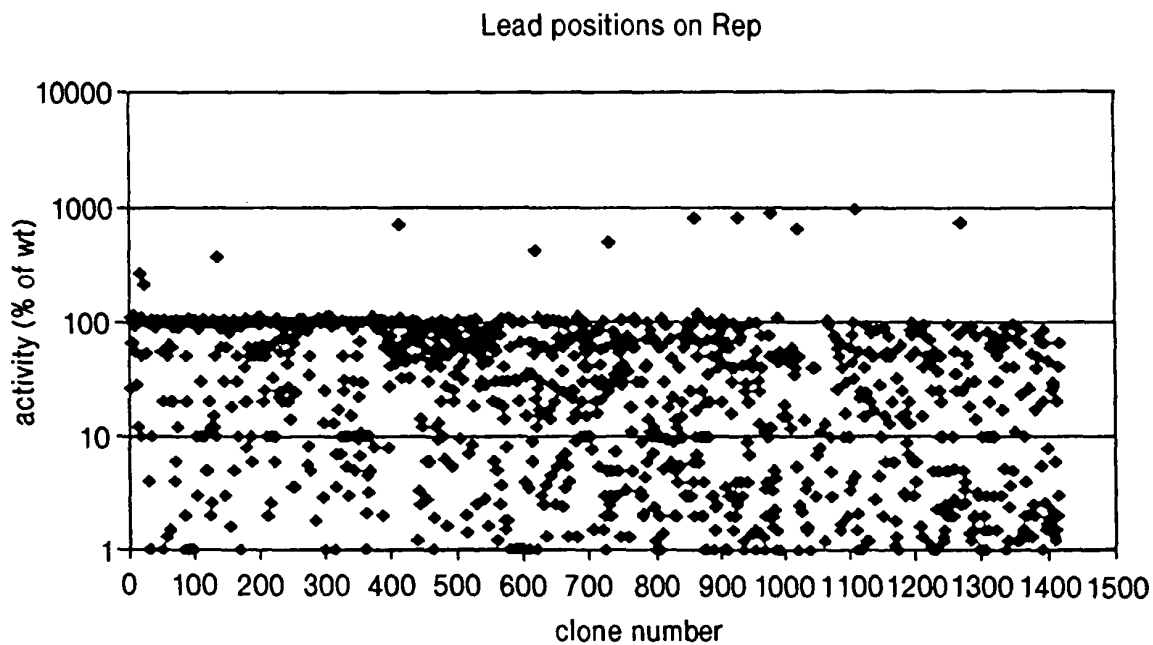


FIG. 3B

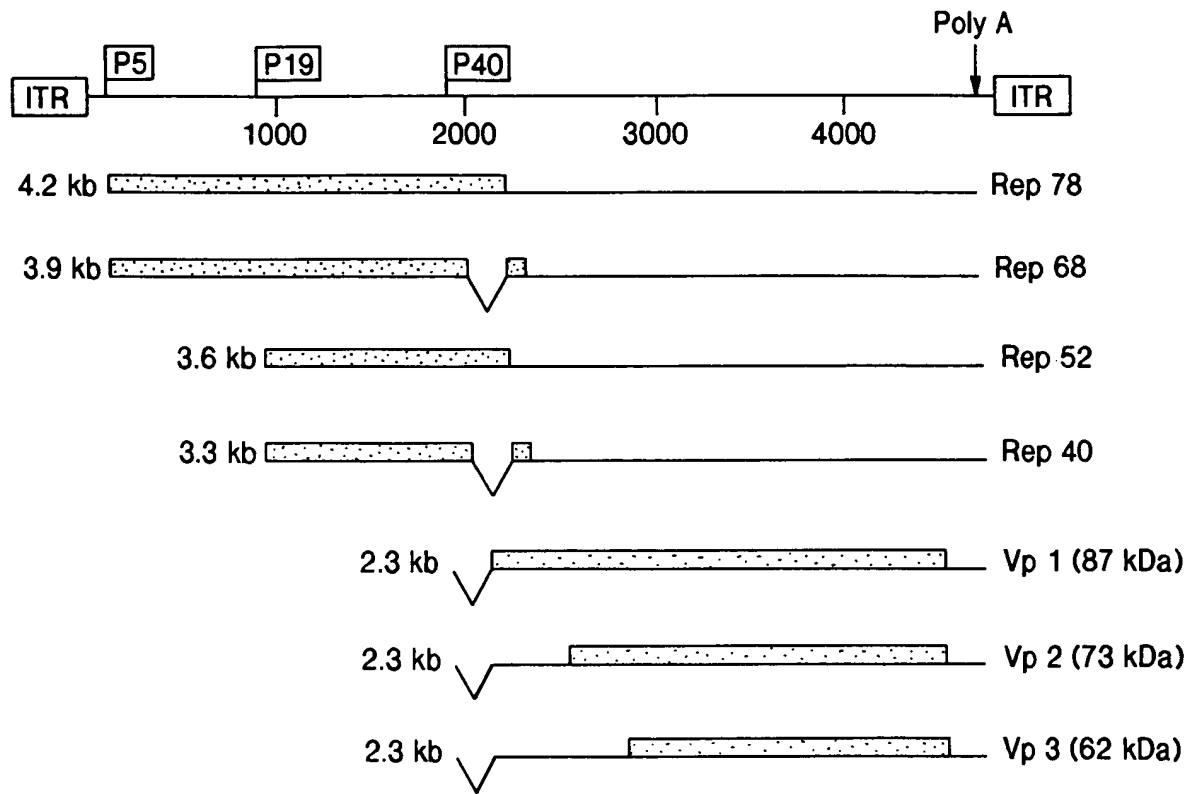


FIG. 4

	10	20	30	40	50	60	
1	MPGFYEIVIKVPSDLDEHLPGISDSFVSWVAEKEWELPPDSMDLNLIEQAPLTVAEKLQ						60
2	MPGFYEIVIKVPSDLDEHLPGISDSFVNWVAEKEWELPPDSMDLNLIEQAPLTVAEKLQ						60
3	MPGFYEIVLKVPSDLDEHLPGISNSFVNWVAEKEWELPPDSMDPNLIEQAPLTVAEKLQ						60
4	MPGFYEIVLKVPSDLDEHLPGISNSFVNWVAEKEWELPPDSMDPNLIEQAPLTVAEKLQ						60
5	MPGFYEIVLKVPSDLDEHLPGISDSFVSWVAEKEWELPPDSMDLNLIEQAPLTVAEKLQ						60
6	MPGFYEIVIKVPSDLDEHLPGISDSFVNWVAEKEWELPPDSMDLNLIEQAPLTVAEKLQ						60
7	MATFYEIVIRVPFDVEEHLPGISDSFVDWVTGQIWELPPESDLNLTVEQPQLTVADRIR						60
C	M**FYE** : *VP*D***HLPGIS+SFV:WV****WELPP*SD***L*EQ**LTVA****						
	70	80	90	100	110	120	
1	RDFLVQWRRVSKAPEALFFVQFEKGESYFHLHLIVETTGVKSMVLGRFLSQIRDKLVQTI						120
2	RDFLVQWRRVSKAPEALFFVQFEKGESYFHLHLIVETTGVKSMVLGRFLSQIRDKLVQTI						120
3	REFLVEWRRVSKAPEALFFVQFEKGETYFHLHLVLIETIGVKSMVVGGRYVSQIKEKLVTRI						120
4	REFLVEWRRVSKAPEALFFVQFEKGETYFHLHLVLIETIGVKSMVVGGRYVSQIKEKLVTRI						120
5	REFLVEWRRVSKAPEALFFVQFEKGDYFHLHLIVETVGKSMVVGGRYVSQIKEKLVTRI						120
6	RDFLTEWRRVSKAPEALFFVQFEKGESYFHMHLVETTGVKSMVLGRFLSQIREKLIQRI						120
7	RVFLYEWNKFSKQ-ESKFFVQFEKGEYFHLHTLVETSGISSMVLGRYVSQIRAQLVKV						119
C	R:FL++W***SK**E**FFVQFEKG+:YFH*H:L+ET:G**SMV:GR::SQI::L*::*						
	130	140	150	160	170	180	
1	YRGIEPTLPNWFVAVTKTRNGAGGGNKKVDDCYIPNYLLPKTQPELQWAWTNMEEYISACL						180
2	YRGIEPTLPNWFVAVTKTRNGAGGGNKKVDDCYIPNYLLPKTQPELQWAWTNMEEYISACL						180
3	YRGVEPQLPNWFVAVTKTRNGAGGGNKKVDDCYIPNYLLPKTQPELQWAWTNMDQYLSACL						180
4	YRGVEPQLPNWFVAVTKTRNGAGGGNKKVDDCYIPNYLLPKTQPELQWAWTNMDQYLSACL						180
5	YRGVEPQLPNWFVAVTKTRNGAGGGNKKVDDCYIPNYLLPKTQPELQWAWTNMDQYISACL						180
6	YRGIEPTLPNWFVAVTKTRNGAGGGNKKVDDCYIPNYLLPKTQPELQWAWTNMEEYISACL						180
7	FOGIEPQINDWVAITKVKK--GGANKVVDSDGYIPAYLLPKVQPELQWAWTNLDEYKLAAL						177
C	**G:EP:***W*A*TK*****GG*NKVVD:YIP*YLLPK*QPELQWAWTN*:Y:*A*L						
	190	200	210	220	230	240	
1	NLAERKRLVAQHLTHVSQTQEQNKENLNPNSDAPVIRSKTSARYMELVGWLVDRGITSEK						240
2	NLAERKRLVAHDLTHVSQTQEQNKENLNPNSDAPVIRSKTSARYMELVGWLVDRGITSEK						240
3	NLAERKRLVAQHLTHVSQTQEQNKENQNPNSDAPVIRSKTSARYMELVGWLVDRGITSEK						240
4	NLAERKRLVAQHLTHVSQTQEQNKENQNPNSDAPVIRSKTSARYMELVGWLVDRGITSEK						240
5	NLAERKRLVAQHLTHVSQTQEQNKENQNPNSDAPVIRSKTSARYMELVGWLVDRGITSEK						240
6	NLTERKRLVAQHLTHVSQTQEQNKENQNPNSDAPVIRSKTSARYMELVGWLVDKGITSEK						240
7	NLEERKRLVAQFLAESSQRS-QEAASQREFSADPVIKSKTSQKYMALVNWLVEHGITSEK						236
C	NL+ERKRLVA**L***SQ***Q*****S**PVI*SKTS**YM*LV*WLV**GITSEK						
	250	260	270	280	290	300	
1	QWIQEDQASYISFNAASNSRSQIKAALDNAGKIMALTSAAPDYLVGPPADIKTNRIYR						300
2	QWIQEDQASYISFNAASNSRSQIKAALDNAGKIMALTSAAPDYLVGPPADIKTNRIYR						300
3	QWIQEDQASYISFNAASNSRSQIKAALDNASKIMSLTKTAPDYLVGSNPPEDITKNRIYQ						300
4	QWIQEDQASYISFNAASNSRSQIKAALDNASKIMSLTKTAPDYLVGSNPPEDITKNRIYQ						300
5	QWIQEDQASYISFNAASNSRSQIKAALDNASKIMSLTKTAPDYLVGQNPPEISSNRIYR						300
6	QWIQEDQASYISFNAASNSRSQIKAALDNAGKIMSLTKTAPDYLVGQPPVEDISSNRIYK						300
7	QWIQENQESYLSFNSTGNSRSQIKAALDNATKIMSLTKSAVDYLVGSSVPEDISKNIWQ						296
C	QWIQE*Q*SY*SFN***NSRSQIKAALDNA:KIM+LTK:A*DYLVG::**+DI::NRI*:						
	310	320	330	340	350	360	
1	I LELNGYEPAYAGSVFLGWAQKRFGKRNTIWLFGPATTGKTNIAEAIHAHVPPFYGCVNWT						360
2	I LELNGYDPAYAGSVFLGWAQKRFGKRNTIWLFGPATTGKTNIAEAIHAHVPPFYGCVNWT						360
3	I LELNGYDPQYAASVFLGWAQKKFGKRNTIWLFGPATTGKTNIAEAIHAHVPPFYGCVNWT						360
4	I LELNGYDPQYAASVFLGWAQKKFGKRNTIWLFGPATTGKTNIAEAIHAHVPPFYGCVNWT						360
5	I LEMNGYDPQYAASVFLGWAQKKFGKRNTIWLFGPATTGKTNIAEAIHAHVPPFYGCVNWT						360
6	I LELNGYDPQYAASVFLGWATKKFGKRNTIWLFGPATTGKTNIAEAIHAHVPPFYGCVNWT						360
7	I FEMNGYDPAYAGSILYGWCQRSEFNKRNTVWLYGPATTGKTNIAEAIHAHVPPFYGCVNWT						356
C	I *E+NGY*P:YA:S***GW***:F*KRNT*WL*GPATTGKTNIAEAIHAHVPPFYGCVNWT						

FIG. 5A

370 380 390 400 410 420
1 NENFPFNDCVDKMWIWEWGKMTAKVVESAKAILGGSKVRVDQCKSSAQIDPTPVIVTS 420
2 NENFPFNDCVDKMWIWEWGKMTAKVVESAKAILGGSKVRVDQCKSSAQIDPTPVIVTS 420
3 NENFPFNDCVDKMWIWEWGKMTAKVVESAKAILGGSKVRVDQCKSSAQIEPTPVIVTS 420
4 NENFPFNDCVDKMWIWEWGKMTAKVVESAKAILGGSKVRVDQCKSSAQIEPTPVIVTS 420
5 NENFPFNDCVDKMWIWEWGKMTAKVVESAKAILGGSKVRVDQCKSSAQIDPTPVIVTS 420
6 NENFPFNDCVDKMWIWEWGKMTAKVVESAKAILGGSKVRVDQCKSSAQIDPTPVIVTS 420
7 NENFPFNDCVDKMLIWEWGKMTNKVVESAKAILGGSKVRVDQCKSSVQIDSTPVIVTS 416
C NENFPFNDCVDKM*IWEWGKMT*KVVESAKAILGGSKVRVDQCKSS*QI+*TPVIVTS
430 440 450 460 470 480
1 NTNMCVIDGNSTTFEHQQPLQDRMFKFELTRRLEHDFGKVTQKEVKEFFRWAQDHVTEV 480
2 NTNMCVIDGNSTTFEHQQPLQDRMFKFELTRRLEHDFGKVTQKEVKEFFRWAQDHVTEV 480
3 NTNMCVIDGNSTTFEHQQPLQDRMFKFELTRRLDHDGKVTQKEVKDFFRWASDHVTDV 480
4 NTNMCVIDGNSTTFEHQQPLQDRMFKFELTRRLDHDGKVTQKEVKDFFRWASDHVTDV 480
5 NTNMCVIDGNSTTFEHQQPLQDRMFKFELTKRLEHDFGKVTQKEVKDFFRWASDHVTEV 480
6 NTNMCVIDGNSTTFEHQQPLQDRMFKFELTRRLDHDGKVTQKEVKDFFRWAKDHVVEV 480
7 NTNMCVVVDGNSTTFEHQQPLEDRMFKFELTKRLPPDFGKITQKEVKDFFAWAKVNQVPV 476
C NTNMC*V*DGNSTTFEHQQPL*DRMFKFELT+RL:*DFGK*TKQEVK+FF*WA:****:V
490 500 510 520
1 AHEFYVRKGGANKRPAPDDADKSEPKRA-----CPSVADPSTSDAEG 522
2 AHEFYVRKGGANKRPAPDDADKSEPKRA-----CPSVADPSTSDAEG 522
3 AHEFYVRKGGAKKR PASNDADVSEPKRQ-----CTSLAQPTTSDAEA 522
4 AHEFYVRKGGAKKR PASNDADVSEPKRQ-----CTSLAQPTTSDAEA 522
5 THEFYVRKGGARKRPAPNDADISEPKRA-----CPSVAQPSTSDAEA 522
6 EHEFYVKKGAKKR PAPSDADISEPKRV-----RESVAQPSTSDAEA 522
7 THEFKVPRELAGTKGAEKSLKRPLGDTVNTSYKSLEKRARLSFVPETPRSSDVTVDPAPL 536
C :HEF*V+***A:***A:****.*****: +:****:***A*:
530 540 550 560 570 580
1 APVDFADRYQNKCSRHAGMLQMLFPCKTCERMNQNFNICFTHGTRDCSECFP--GVSESEQ 580
2 APVDFADRYQNKCSRHAGMLQMLFPCKTCERMNQNFNICFTHGTRDCSECFP--GVSESEQ 580
3 P-ADYADRYQNKCSRHVGMNLMFLFPCKTCERMNQISNVCFTHGQRDCGECFPGMSESQPV 581
4 P-ADYADRYQNKCSRHVGMNLMFLFPCKTCERMNQISNVCFTHGQRDCGECFPGMSESQPV 581
5 P-VDYADRYQNKCSRHVGMNLMFLPCRQTCERMNQNVDFICFTHGVMDCAECFP--VSESQPV 580
6 S-INYADRYQNKCSRHVGMNLMFLPCRQTCERMNQNSNICFTHGQKDCLECFP--VSESQPV 579
7 RPLNWNRSRYDCKDYHAQFDNISNKCDECEYLNRGKNGCICHNVTHCQICHG----- 588
C :::+:**RY**KC**H:***:****C::CE**N*:*:C**H*:*:C.*C**...::+:::
590 600 610 620
1 PVVRKRITYRKLCAIHHLGLGRAPEIACSACDLVNVDLDDCVSEQ 623
2 PVVRKRITYRKLCAIHHLGLGRAPEIACSACDLVNVDLDDCVSEQ 623
3 SVVKKKTYQKLCPIHHILGRAPEIACSACDLANVDLDDCVSEQ 624
4 SVVKKKTYQKLCPIHHILGRAPEIACSACDLANVDLDDCVSEQ 624
5 SVVRKRITYQKLCPIHHIMGRAPEVACSACELANVDLDDCDMEQ 623
6 VSVVKKAYQKLCYIHHIMG-KVPDACTACDLVNVDLDDCIFEQ 621
7 -----IPPWEKENLSDFGDFDDANKEQ 610
C :+*:+:*:*:***:***:****+*:*:***D*DD*:*:EQ

FIG. 5B